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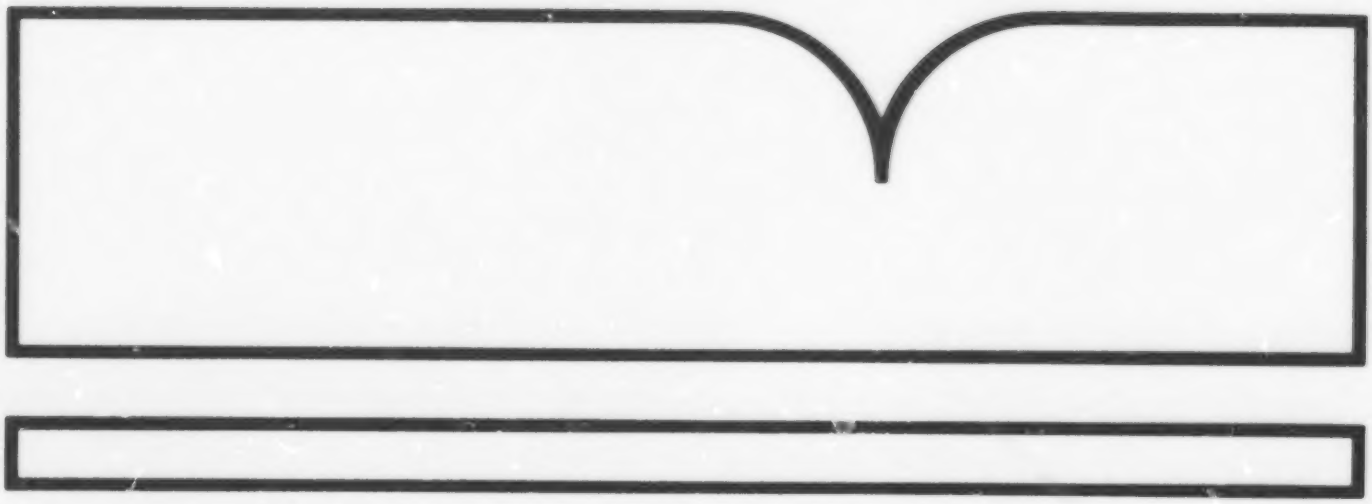

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16. ABSTRACT

We have found that a single dose of amitraz, a formamidine pesticide, produces death in mice which begins about two days after dosing and continues for up to 5 more days. To further examine this phenomenon, adult albino mice of both sexes were treated with either yohimbine (10 mg/kg, i.p.) or deionized water (6 ml/kg, i.p.), immediately before an injection of amitraz (600 mg/kg, i.p.) and twice daily thereafter for eight days. Male mice treated with water were more susceptible than water-treated females to the lethal effects of amitraz. In addition, yohimbine treatment significantly decreased the number of deaths in both sexes. These data suggest that an 2-adrenergic mechanism is involved in the delayed lethaltiy produced by amitraz.

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YOHIMBINE ATTENUATES THE DELAYED LETHALITY INDUCED IN MICE BY AMITRAZ, A FORMAMIDINE PESTICIDE

(Amitraz; α2-adrenergic receptors; formamidine pesticides; lethality in mice)

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SUMMARY

We have found that a single dose of amitraz, a formamidine pesticide, produces death in mice 2–5 days after dosing. To further examine this phenomenon, adult albino mice of both sexes were treated with either yohimbine (10 mg/kg, i.p.) or deionized water (6 ml/kg, i.p.), immediately before an injection of amitraz (500 mg/kg, i.p.) and twice daily thereafter for 8 days. Male mice treated with water were more susceptible than water-treated females to the lethal effects of amitraz. In addition, yohimbine treatment significantly decreased the number of deaths in both sexes. These data suggest that an α2-adrenergic mechanism is involved in the delayed lethality produced by amitraz.

INTRODUCTION

Amitraz (AMZ) is a member of the formamidine class of acaricide/insecticide which is used world-wide in agriculture and in veterinary medicine. Since it is considered relatively non-toxic, few studies have examined its effects in mammals. One study [1] has reported long-lasting (7–10 days) toxic manifestations in rats and delayed deaths occurring 2–6 days after dosing with a commercial product containing amitraz and xylene. The acute oral LD_{50} value in mice has been stated to be 1600 mg/kg [2]. In the course of ongoing research in this laboratory, we observed that

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2 mice which had been dosed with AMZ (600 mg/kg) were found dead 5 days later. Delayed mortalities, in the order of days, are relatively unusual in pesticide research. Thus, we undertook a series of experiments to characterize this action, by determining sex-related differences and whether the lethality could be blocked with yohimbine, an α2-adrenergic antagonist, which has recently been reported to reverse several other effects of amitraz [3,4].

MATERIALS AND METHODS

Animals

Adult CD-1 mice were obtained from Charles River Breeding Laboratories (Portage, MI), 2–5 weeks before testing began. Male mice were housed individually, and females were housed in groups of 4–6, in plastic cages (28.5 × 17.5 × 12 cm) with pine shaving bedding and wire mesh tops. Food (Wayne Lab-Blox, Peoria, IL) and tap water were available ad lib. Mice were kept in the laboratory, which was maintained at 21–24°C with regular work-day lighting conditions.

Experiment 1

15 Male mice were injected i.p. with AMZ (600 mg/kg) on day 0. These mice were tested in motor activity measurement devices for 25 min daily, as part of another pilot study. The number of dead mice was recorded daily. One subject was autopsied for gross abnormalities immediately after death.

Experiment 2

Male and female mice were separated into 2 treatment groups designated to receive injections of either yohimbine (10 mg/kg) or deionized water (6 ml/kg). Group sizes were: yohimbine-treated males, 12; water-treated males, 9; yohimbine-treated females, 15; water-treated females, 14. On day 0, at approx. 08:00, all mice were injected i.p. with the appropriate treatment (yohimbine or water), followed 30 min later by AMZ (600 mg/kg). Thereafter, they received injections of water or yohimbine at about 16:00 and 08:30 daily for 8 days. Deaths were recorded daily and body weights were taken 2–3 times a week.

Experiment 3

16 Mice of each sex were separated into 2 equally sized groups which received injections of either yohimbine (10 mg/kg) or water, twice daily at 08:30 and 16:30 for 7 days. Body weights were taken 2–3 times a week and deaths were recorded daily.

Chemicals

AMZ (97% purity) was generously supplied by Nor-Am Chemical Co. (Wilmington, DE), and yohimbine HCl was purchased from Sigma Chemical Co. (St. Louis, MO). Yohimbine was dissolved in deionized water, and the dose refers to the HCl salt. A new solution was prepared daily. AMZ was dissolved in 95% ethanol

and suspended with Emulphor (EL-620, GAF Corp., New York, NY) and isotonic saline such that the final concentrations of ethanol and Emulphor were 5% each. For Expt. 1, AMZ was given in a volume of 3 ml/kg body wt, and for Expt. 2 AMZ and yohimbine were given at 6 ml/kg body wt.

RESULTS

Experiment 1

Fig. 1 shows the daily cumulative deaths for 15 mice. The first death occurred on day 3, i.e., the third day after dosing. By day 7, 80% (12 of 15) of the mice had died. Results of the autopsy indicated an enlarged, impacted intestine and cecum.

Experiment 2

The cumulative deaths in yohimbine-treated and water-treated mice are shown in Fig. 2. Males treated with water were significantly more sensitive than water-treated females, with 89% (8 of 9) males and 50% (7 of 14) females dead by the fifth day (Fisher's exact test, $P<0.05$; [5]). In both sexes, yohimbine significantly decreased mortality, to 42% (5 of 12) in yohimbine-treated males and 20% (3 of 15) in yohimbine-treated females. Body weights of surviving mice are shown in Fig. 3. A large weight loss was observed in all subjects. Weight loss of the mice succumbing to AMZ was not different from that of surviving mice (these data are excluded from

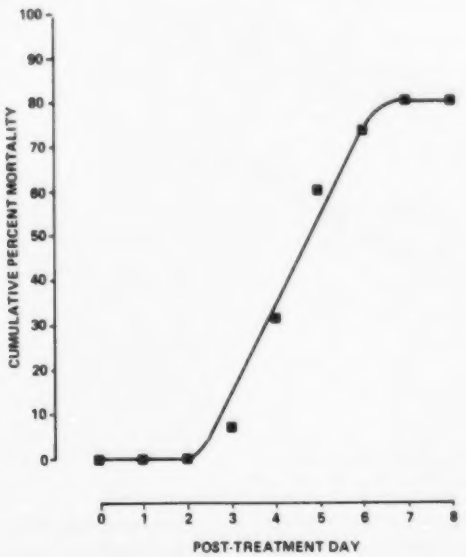

Fig. 1. Cumulative % mortality in 15 male mice dosed with AMZ (600 mg/kg) on day 0. The curve is fit by eye.

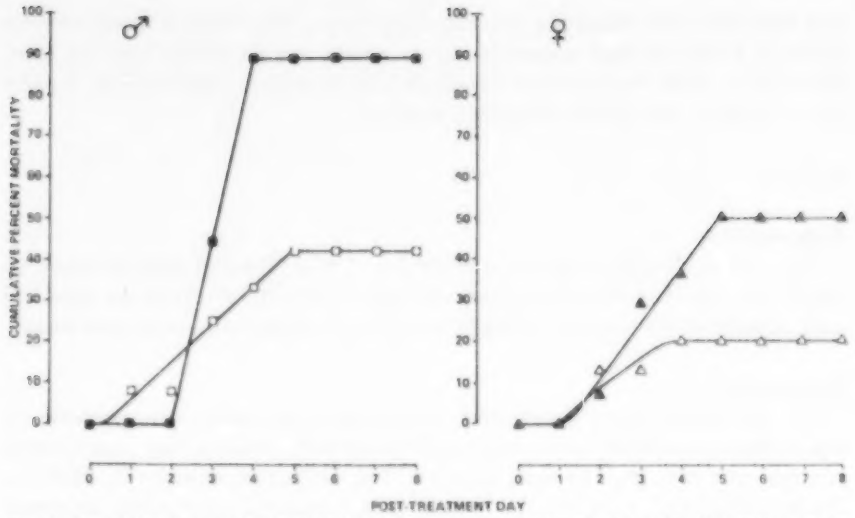

Fig. 2. Cumulative % mortality in male (□) and female (△) mice dosed with AMZ (600 mg/kg) on day 0, and treated with either water (filled symbols) or yohimbine (10 mg/kg; open symbols) twice daily for 8 days. The curves are fit by eye.

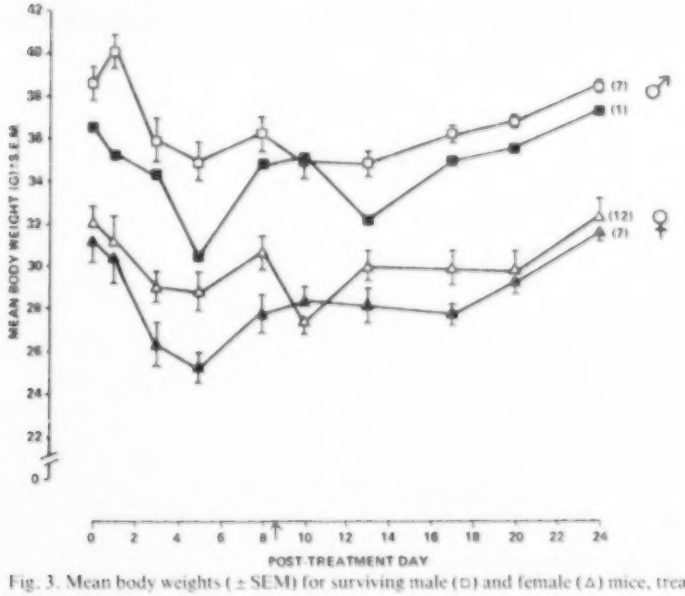

Fig. 3. Mean body weights (± SEM) for surviving male (□) and female (△) mice, treated with either water (filled symbols) or yohimbine (10 mg/kg; open symbols) twice daily for the first 8 days. Number in parentheses indicate number of subjects in each group; arrow indicates when daily injections were discontinued.

the figure for clarity). However, average weights of yohimbine-treated mice were usually greater than or equal to weights of water-treated mice. For all groups, 24 days were required for weights to return to pre-dosing levels.

Experiment 3

No deaths were produced by twice daily injections of yohimbine (10 mg/kg) in either sex. This treatment did produce a drop in body weights, but the magnitude was not as great (only 1–3 g decrease in average body weights) as was obtained with the AMZ-treated mice (4–6 g average weight loss). In addition, weights returned to pre-dosing levels within one day after injections were discontinued.

DISCUSSION

The delayed lethality produced by AMZ is highly reproducible. There was a distinct sex difference in the magnitude, but not in the time course of mortality. This sex difference could be due to a number of factors, including different rates of liver metabolism, distribution into body fat, or hormonal influences. Another possibility is attenuation of AMZ-induced hypothermia in the group-housed females; however, a warm environment was ineffective in decreasing toxicity in rats [1]. There were never any signs of CNS stimulation (e.g., convulsions) which are evident following lethal doses of chlordimeform (another formamidine pesticide to which AMZ is often likened). In fact, this dose of AMZ only produced mild ataxia and sedation, from which the mice apparently recovered within hours. The prolonged effects on mortality and especially on body weights, which took over three weeks to recover, are not in agreement with the report that AMZ and its primary metabolite, BTS-27271, are rapidly eliminated [6]. In fact, these data suggest either a very slow excretion of the parent compound and/or its active metabolites, or a long-term change in some properties of the target organs.

Turnbull [1] reported that in rats there was no antidote for AMZ-produced lethality, after using a variety of pretreatments, e.g., diuretics, phentolamine, and nikethamide. This paper is therefore the first report of a successful antagonism of the delayed lethal effects of AMZ, and suggests the use of yohimbine in antidotal therapy for cases of over-exposure.

Several gastrointestinal functions, including motility, are mediated by α2-adrenoceptors,and α2-agonists slow motility and produce constipation which can be blocked by yohimbine and other α2-antagonists [7,8]. Reports from Australia indicate that AMZ produces a dose-related, severe intestinal stasis in horses sprayed for tick control [9,10]. AMZ has been shown to lower blood pressure and heart rate in rats via α2-stimulation [3], and most recently Hsu and Lu [4] reported an AMZ-induced delayed gastrointestinal transit in mice which was also mediated by α2-receptors. This evidence along with the present report raises suspicion that the high dose of AMZ is producing a profound intestinal stasis, leading to general debilitation and eventual death. We speculate whether a more frequent

yohimbine dosing regimen (e.g., around the clock) to produce steady-state blood levels would totally prevent AMZ-induced delayed lethality. Another piece of evidence suggesting an action of AMZ on gut motility is the report that, in vitro, AMZ inhibits contractions of the guinea pig ileum induced by histamine H-1 agonists [12]. Thus AMZ may have a direct action on H-1 receptors as well as α2-receptors, or there may exist an interplay of all these systems mediating gut contractions. Of course, there may be other contributing factors. Yohimbine has been reported to have other actions as well as α2-adrenergic, such as blockade of postsynaptic dopamine receptors [11]. However, the available data suggest that the delayed lethality produced by AMZ is at least partially mediated by α2-mechanisms.

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